Widespread exploitation of the honeybee by early Neolithic farmers

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The pressures on honeybee (*Apis mellifera*) populations, resulting from threats by modern pesticides, parasites, predators and diseases, have raised awareness of the economic importance and critical role this insect plays in agricultural societies across the globe. However, the association of humans with *A. mellifera* predates post-industrial revolution agriculture, as evidenced by the widespread presence of ancient Egyptian bee iconography dating to the Old Kingdom (approximately 2400 BC)[1]. There are also indications of Stone Age people harvesting bee products; for example, honey hunting is interpreted from rock art[2] in a prehistoric Holocene context and a beeswax find in a pre-agriculturalist site[3]. However, when and where the regular association of *A. mellifera* with agriculturalists emerged is unknown[4]. One of the major products of *A. mellifera* is beeswax, which is composed of a complex suite of lipids including *n*-alkanes, *n*-alkanoic acids and fatty acyl wax esters. The composition is highly constant as it is determined genetically through the insect's biochemistry. Thus, the chemical 'fingerprint' of beeswax provides a reliable basis for detecting this commodity in organic residues preserved at archaeological sites, which we now use to trace the exploitation by humans of *A. mellifera* temporally and spatially. Here we present secure identifications of beeswax in lipid residues preserved in pottery vessels of Neolithic Old World farmers. The geographical range of bee product exploitation is traced in Neolithic Europe, the Near East and North Africa, providing the palaeoecological range of honeybees during prehistory. Temporally, we demonstrate that bee products were exploited continuously, and probably extensively in some regions, at least from the seventh millennium cal BC, likely fulfilling a variety of technological and cultural functions. The close association of *A. mellifera* with Neolithic farming communities dates to the early onset of agriculture and may provide evidence for the beginnings of a domestication process.

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The honeybee holds a unique place in human culture. Notwithstanding its present-day economic importance, it has been revered over the millennia for the sheer beauty and complexity of the social organization within its colonies. For these reasons the honeybee is the most researched of the social insects, with its origin being regularly considered[5]. The last Ice Age would have had a major effect on the honeybee with the ice sheets restricting European populations to the northern Mediterranean hinterlands[6]. With the glacial retreat, the population would have subsequently expanded northwards. However, due to the lack of a Holocene fossil record[7], the honeybee is ecologically invisible for most of the past 10,000 years.

Intriguingly, this is the period during which Neolithic agriculture emerged and spread out of southeastern Anatolia and the Levant, with some human population movement into ecological zones also conducive to the honeybee. Indeed, progressive woodland clearances by pioneer prehistoric farmers may have opened up forests, favouring light-demanding shrubs, herbs and fruit trees (for example, *Rosaceae*)[8]. Whether this would have exerted negative or positive effects on honeybee populations is unknown[8,9]. Given the latter, an opportunity exists to investigate the presence and early exploitation of the honeybee by prehistoric farming communities through the cultural materials recovered from Neolithic sites, namely their recently invented pottery vessels, and in doing so, to assess the palaeoecological range of the honeybee in the Holocene.

Although the most obvious reason for exploiting the honeybee would be for honey, a rare source of sweetener for prehistoric people, beeswax would likely have been an equally important material for various technological, ritual, cosmetic and medicinal applications[10]. Indeed, beeswax has been regularly detected in later archaeological and historic periods in lipid extracts from the fabric of unglazed pottery vessels[11] where it is assumed to be a residue of honey use in cooking, or from the use of vessels for processing wax combs[12–14], with beeswax being absorbed through repeated contacts. Beeswax has also been detected as a fuel in lamps and in larger vessels used as proto-beehives, for example Roman Greece (second century BC to fourth century AD)[15,16] and applied as a post-firing treatment to waterproof vessels[17].

The detection of beeswax in archaeological and historic contexts rests on its complex chemistry providing a unique and relatively recalcitrant chemical signature. Fresh beeswax comprises a complex mixture of aliphatic compounds consisting of series of homologues differing in chain-length by two methylene groups[18]. Medium-chain *n*-alkanes range from C_{23} to C_{31} (with C_{27} dominating in *A. mellifera*), and *n*-alkanoic acids from C_{20} to C_{36}. Monoesters comprise predominantly alkyl palmitates (C_{38} to C_{52}), with characteristic hydroxyl monoesters comprising long-chain alcohols (C_{24} to C_{38}) esterified mainly to hydroxypalmitic acid, ranging between C_{40} and C_{54} (ref. 18). The hydrophobic nature of beeswax makes it relatively resistant to degradation. Hence, if protected from extensive microbial attack and/or exposure to high temperatures during anthropogenic manipulation, the aforementioned chemical characteristics can be used in assessing its presence[10,19] (Figs 1 and 2).

Adopting this lipid biomarker approach, we now explore the association of the honeybee with the spread of early Old World farmers based on lipid residue analyses of more than 6,400 pottery vessels (Supplementary Information sections 1 and 2). Combining our new findings with published occurrences of beeswax in prehistoric pottery allows the association between honeybees and early farmers to be mapped spatially and temporally through prehistory (Figs 3 and 4).

The oldest evidence for beeswax comes from Neolithic sites in Anatolia dating from the seventh millennium cal BC, as these sites are the locations of the oldest pottery vessels in Europe and Eurasia. Most of the assemblages investigated comprised globular or bowl shape 'cooking' vessels, an interpretation supported by the finding of ruminant and porcine animal fats in significant numbers of vessels. No beeswax residues were detected during the intensive investigations of > 380 vessels from the Levant, although only 34 residues were detected[20]. Moving into eastern Anatolia, the site of Çayönü Tepesi revealed two beeswax residues from 83 vessels from the seventh millennium including an exceptionally well-preserved residue containing all the biomarkers of beeswax (Fig. 1b–f). The free *n*-alkanols, dominated by C_{30} and C_{32} homologues, do not occur in fresh beeswax but are a feature of aged wax, due to hydrolysis of the wax esters. The high abundance of $C_{18:0}$ fatty acid suggests mixing with mammalian animal fat, the latter being common in other sherds in the assemblage[20]. The second sherd from this site contained a lower concentration of beeswax but all the biomarkers were clearly evident. These two residues establish the easterly limit of the beeswax detected in this investigation and provide the oldest unequivocal evidence, to our knowledge, of honeybee exploitation by early Neolithic farmers.

In central Anatolia, extensive investigations of organic residues in 650 vessels, mainly from the site of Çatalhöyük, revealed abundant animal fat residues. Only one residue showed tentative evidence for beeswax based on wax esters, dominated by C_{46} and C_{48} homologues; however, the *n*-alkanols do not exhibit the familiar distribution. *n*-Alkanes were detectable but the distribution is skewed towards the higher homologues compared to that expected in fresh beeswax, although such distributional changes are frequently seen in historical and archaeological beeswax, assumed to arise by sublimation during ageing or heat treatment[10]. The tentative identification of this very early beeswax residue at Çatalhöyük is supported by the discovery of a striking depiction of a honeycomb-like pattern painted on a wall at the site[21].

Analyses of approximately 570 cooking vessels from northwestern Anatolia revealed 72 lipid residues of which 4 were identified as containing beeswax, from Aşağı Pinar and Toptepe, dating to 5500–5000 cal BC. Although the overall purity of the beeswax (two were mixed with ruminant fat) and lipid concentrations (20 to 220 μ g per gram of sherd) were quite variable, the distributions were unmistakable. One of the beeswax finds from Toptepe is well preserved, albeit with ageing evident from the hydrolytically released free *n*-alkanols and slight distortions of the various homologous series, through loss of lower homologues.

The most abundant evidence for honeybee exploitation by early farmers was seen in the rest of the Balkan Peninsula. The full range of beeswax biomarkers was identified in sherds from bowls, pans and

sieves from the Late Neolithic sites of Paliambela, Greece (4900–4500 cal BC), Măgura, Romania (Fig. 2a; 5500–5200 cal BC) and Drenovac Turska Česma, Serbia (5300–4700/4600 cal BC). A large number of beeswax residues were found in Neolithic potsherds (11 residues out of 81 sherds analysed) from Attica, the Peloponnese and the Cyclades (Aegean Islands), dating between 5800 and 3000 cal BC, firmly establishing the long tradition of bee exploitation in this region. Overall, the incidence of beeswax residues is highest in the Balkan Peninsula, where of the 1,915 Neolithic sherds analysed, 473 yielded lipid residues, of which 5.5% contained beeswax.

In Central Europe, pure beeswax was recovered from potsherds from *Linearbandkeramik* (LBK) sites occupied by the earliest farmers of Austria and Germany (oldest LBK) including the sites of Brunn am Gebirge (5500–5400 cal BC) and Niederhummel (5360–5220 cal BC), pushing back the date for bee exploitation in this region by approximately 1,500 years[13] (Fig. 2b). Beeswax was also detected in late sixth millennium LBK sites of Ludwinowo 7 and Wolica Nowa, Poland[17]. In France, the exploitation of bee products is evident during the second half of the fifth millennium at *Chasséen* sites (Font-Juvénal, Chassey-le-Camp and Bercy[10]) and fourth millennium at the Lake Village sites of Clairvaux-les-Lacs (3900 to 3700 BC) and Chalain 3 (ref. 22) and 4 (3200 to 3100 BC and 3040 to 2990 BC). High incidence of beeswax (approximately 15% of the detectable residues) was identified in fifth millennium sherds from two Slovenian sites (Ajdovska jama and Moverna vas)[23].

Around 130 sherds have so far been analysed from the Iberian Peninsula. However, no beeswax residues have yet been detected, although the overall preservation of organic residues was poor. Further investigations will likely reveal examples of beeswax in Neolithic pottery from this region.

The northerly limit of bee exploitation in northern Europe appears to be Denmark with two beeswax finds in late Mesolithic and Neolithic contexts[24]. Around 5° to the south in southern Britain, beeswax is evident in 7 vessels amongst the approximately 670 Neolithic vessels analysed. These findings clearly counter any arguments for a late introduction of the honeybee into the British Isles[8,25]. Interestingly, however, investigations of nearly 1,200 Mesolithic and Neolithic vessels from Ireland, Scotland and Fennoscandia[26,27] have failed to reveal any conclusive evidence of beeswax (Supplementary Information section 3). Given that organic residue preservation in these regions is excellent, the lack of beeswax would seem to establish the ecological limit of *A. mellifera* at that time. Similar arguments are likely to account for the absence of beeswax residues from >350 prehistoric pottery vessels from the Eurasian Steppe[28].

Finally, we report the first evidence for bee exploitation by Neolithic pastoralists in North Africa. The analysis of 71 sherds from the Algerian site of Gueldaman revealed a single well-preserved beeswax residue (fifth millennium BC). The preservation is again exceptional with *n*-alkanes, *n*-fatty acids and fatty acyl wax ester distributions providing an unequivocal identification of beeswax. The presence of free long chain *n*-alkanols and lack of hydroxy fatty acid wax esters are indicative of diagenesis and/or use-related alteration. However, the overall distribution indicates the wax residue derives from *A. mellifera* (Fig. 2c).

In conclusion, the approximately 50 new finds of beeswax residues considered above provide evidence for the widespread exploitation of the honeybee by the early agriculturalists and pastoralists of the Near East, Europe and North Africa dating back nearly 9,000 years (Fig. 3). In all these regions the new data have either provided the first evidence of honeybee exploitation in a region, as in North Africa, or pushed the chronology of human–honeybee association to substantially earlier dates, as in Anatolia and Central Europe (Fig. 3b). The lack of evidence for beeswax use at Neolithic sites north of the 57th parallel North may suggest an ecological limit to the natural occurrence of honeybees. Indeed, harsh high-latitude conditions, even with temperatures warmer than today[29], would affect the foraging capabilities of honeybees[30]. Critically, in the absence of a Holocene fossil record for *A. mellifera*[7] these findings provide the first ancient biomolecule-based palaeoecological map of the distribution of an economically and culturally important animal (Fig. 4).

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Methods

Lipid residue analyses. All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade (typically > 98% of purity).

A sub-sample (1 to 3 g) from archaeological potsherds was cleaned with a modelling drill to remove any exogenous lipids (from the soil and handling) and crushed with a solvent-washed mortar and pestle. An internal standard (*n*-tetratriacontane, typically 20 µ g) was added to the powdered sherd to enable the quantification of lipid extract. Ground samples of sherds were extracted with $CHCl_3$/MeOH (2:1 (vol/vol), 2 × 10 ml) using ultrasonication. Both supernatants were combined and the solvent was removed under a gentle stream of nitrogen at 40 °C. Aliquots of the total lipid extract (TLE) were treated with 40 µ l of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Sigma Aldrich) for 1 h at 70 °C and the BSTFA in excess evaporated under a gentle stream of nitrogen. The trimethysilylated TLE was diluted in hexane (typically 50 to 150 µ l) and submitted to analysis by high-temperature gas chromatography (HTGC) and high temperature gas chromatography-mass spectrometry (HTGC/MS) to identify the major compounds present.

All TLEs were initially screened in a Hewlett-Packard 5890 Series II gas chromatograph equipped with a fused-silica capillary column (15 m × 0.32 mm) coated with dimethyl polysiloxane stationary phase (DB-1HT; film thickness, 0.1 µ m; Agilent Technologies). Derivatized extracts (1.0 µ l) were injected on-column using a cool on-column inlet in track oven mode. The temperature was held isothermally for 2 min at 5 °C and then increased at a rate of 10 °C min^{-1} and held at 350 °C for 10 min. The flame ionization detector (FID) was set at a temperature of 350 °C. Helium was used as a carrier gas and maintained at a constant flow of 4.6 ml min^{-1}. Data acquisition and processing were carried out using the HP Chemstation software (Rev. B.03.02 (341), Agilent Technologies).

HTGC/MS analyses of trimethylsilylated aliquots were performed using a Thermo Scientific Trace 1300 gas chromatograph coupled with an ISQ single quadrupole mass spectrometer. Diluted samples were introduced using a PTV injector in split mode (split flow of 30 ml min^{-1}, split ratio of 6.0) onto a 0.53 mm fused silica pre-column connected to a 15 m × 0.32 mm i.d. fused-silica capillary column coated with dimethyl polysiloxane stationary phase (Rxi-1HT; film thickness, 0.1 µ m; Restek). The initial injection port temperature was 50 °C with an evaporation phase of 0.05 min, followed by a transfer phase from 50 °C to 380 °C at 0.2 °C min−1. The oven temperature was held isothermally for 2 min at 50 °C, increased at a rate of 10 °C min^{-1} to 280 °C, then at a rate of 25 °C min^{-1} to 380 °C and finally held at 380 °C for 5 min. Helium was used as a carrier gas and maintained at a constant flow 5 ml min^{-1}. The mass spectrometer was operated in the electron ionization (EI) mode (70 eV) with a GC interface temperature of 380 °C and a source temperature of 340 °C. The emission current was 50 µ A and the mass spectrometry set to acquire in the range of *m/z* 50–950 Daltons at two scans per second. Data acquisition and processing were carried out using the Thermo XCalibur software (version 3.0.63). Peaks were identified on the basis of their mass spectra, gas chromatography (GC) retention times, by

comparison with the NIST mass spectral library (version 2.0) and by comparison with modern beeswax (from the Loire department, France).

Construction of Fig. 4. The total number of archaeological sites investigated is 166, but only 154 of these fell within the geographical area of interest (longitude − 10° to 42° and from latitude 25° to 62°, see Supplementary Information section 1). To estimate the distribution of beeswax residues in continuous space from irregularly spaced data, linear interpolation was performed in the triangles bounded by data points[32,33]. The output grid was made of 530 × 380 points evenly spaced over the range of latitude and longitude. No extrapolation was being used. Kriging was used to narrow the interpolation values to locations around data points (and not show interpolation values where there is no data). Kriging allows to obtain weights of the prediction locations based on the distance between data points, with lower variance where data points are and higher variance where there is no data. Interpolation, kriging and plotting were all performed in R version 2.15.1 (ref. 34). Interpolation was performed using the function 'interp' from the package 'akima' (CRAN repository, http://cran.r-project.org/web/packages/akima/akima.pdf). Kriging was performed using the function 'krige.conv' from the package 'geoR' (CRAN repository, http://cran.r-project.org/web/packages/geoR/geoR.pdf, further information on the package 'geoR' can be found at http://www.leg.ufpr.br/geoR). R code available upon request to P.G.

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Figure 1

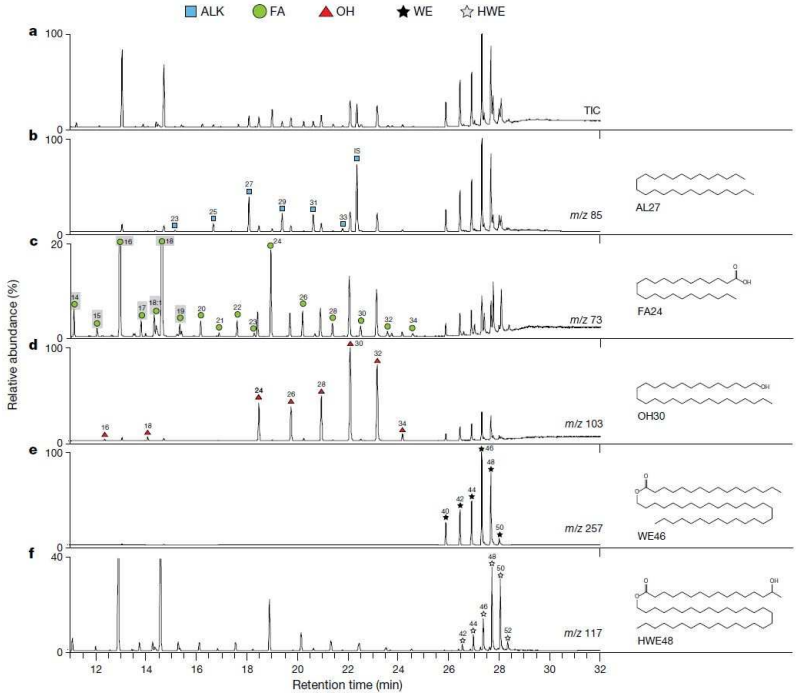
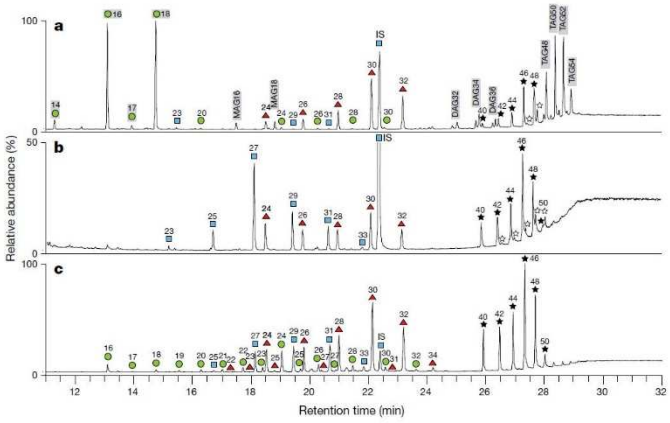
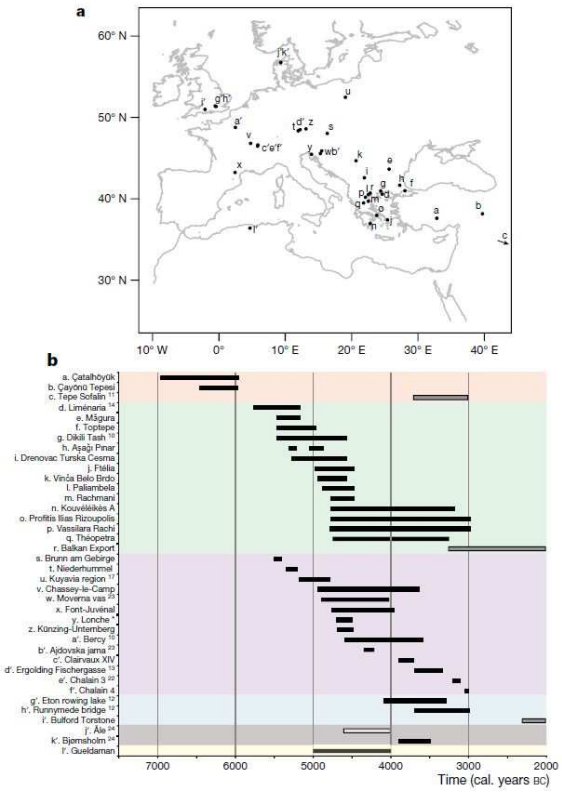


Figure 2



352 **Figure 3**



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354 **Figure 4**

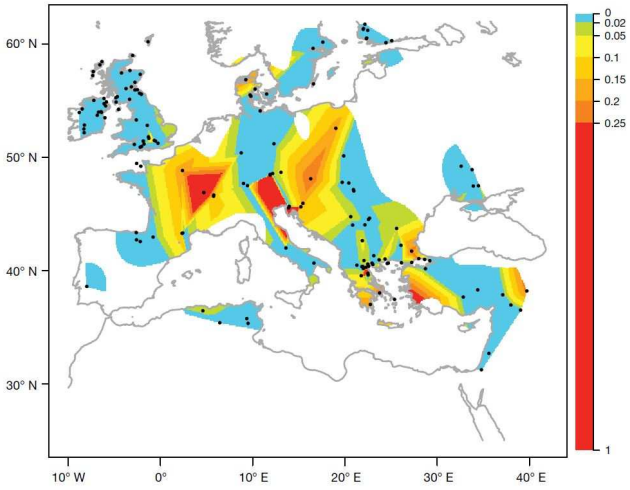

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Figure captions

Figure 1 | **High-temperature gas chromatography/mass spectrometry chromatograms of total lipid extract of a sherd from Çayönü Tepesi (6500–6000 cal BC) containing beeswax. a–f**, Partial total ion current chromatogram (**a**) and mass chromatograms (**b–f**) displaying ion masses of characteristic fragments from the main compound classes comprising the extract (*m/z* 85, 73, 103, 257 and 117, respectively) with the molecular structure of the most abundant component for each compound class. Squares, *n*-alkanes (ALK); circles, *n*-alkanoic acids (fatty acids, FA); triangles, *n*-alkanols (OH); black asterisks, fatty acyl monoesters (WE); grey asterisks, hydroxyl fatty acyl monoesters (HWE); IS, internal standard (*n*-tetratriacontane); number *n* and *n*:*i*, acyl carbon number with zero or *i* degrees of unsaturations. Compounds shown with a grey background are interpreted as originating from mammalian animal fats.

Figure 2 | **Partial gas chromatograms of total lipid extracts from Neolithic sherds from each geographical region. a**, Mağura (5500–5200 cal BC). **b**, Niederhummel (5360–5220 cal BC). **c**, Gueldaman (fifth millennium BC). **a** is interpreted as mixture of animal fats and beeswax; **b** and **c** as pure beeswax. MAG, monoacylglycerols; DAG, diacylglycerols,TAG, triacylglycerols. Other peak attributions as in Fig. 1.

Figure 3 | **Geographical distribution of prehistoric sites in the date range 7500 and 2000 cal BC yielding beeswax residues. a**, Locations of archaeological sites. **b**, Chronology of beeswax use in the Near East, the Balkan Peninsula, mainland Europe, Scandinavia, the UK and northern Africa. Neolithic finds in black, pre-Neolithic (hunter-gatherer contexts) in light grey and Bronze Age in dark grey. * Dental filling re-examined after ref. 31.

Figure 4 | **Regional distribution of beeswax residues in potsherd lipid extracts.** Interpolated map of Old World beeswax occurrences (proportion of beeswax residues per number of residues in pottery sherds, in percentages) during the Neolithic (including the Mesolithic sites available). Colours and colour key show the proportions of beeswax residues estimated by surface interpolation, where collection locations are represented by dots (n = 154).

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Author Contributions M.R.-S., M.R., R.P.E. and A.K.O. conceived and planned the project about beeswax in prehistory. M.R.-S., M.R. and R.P.E. wrote the paper. M.R.-S., M.R., L.J.E.C., O.D., J.D., S.Mil., S.Mir., M.P., J.S., L.S., H.L.W., M.Bart. and D.U.-K. undertook planning of regional lipid residue analyses projects, sampling, analytical work and data analysis. P.G. created Figure 4 and Supplementary Information section 3. All other authors either directed excavations or provided expertise in relation to pottery collections and essential insights into the study region and sites.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. R code is available upon request to P.G. (p.gerbault@ucl.ac.uk). Correspondence and requests for materials should be addressed to M.R.-S. (melanie.salque@bristol.ac.uk), M.R. (martine.regert@cepam.cnrs.fr) or R.P.E. (r.p.evershed@bristol.ac.uk).